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Biometric Study, Content and Physical Characteristics of Volatile Oil in Leaves of Belangu plant (*Lallemantia royleana* L) affected by Foliar and Soil N-P-K Fertilization with Chemical Study of Seeds fixed Oil

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ABSTRACT

Balangu (*Lallemantia royleana* L) is a number of labiatae family and it's consider one of the most important medical plants that used in food and traditional medicine in Iraq . Although the importance of this plant in herbal medicine in Iraq and other countries, there are no studies reported on cultivation or chemical study in Iraq. This study was carried out to investigate the effect of foliar and soil N-P-K fertilization on growth parameters and leaves volatile oil quantity and quality with chemical study of seeds oil composition.

***Keywords*:** Aushari cheese, PCR, RAPD, Lactobacillus Acidophilus Isolated.

1. Introduction

Balangu (*Lallemantia royleana* L) is grow wildy in north of Iraq and neighboring countries such as Turkey and Iran (Chakravarty, 1976). Belango seeds are used as cold infusion in herbal medicine as diuretic, heart stimulant, laxative, cough and external use for carbuncle treatment. Balangu cultivated for its seeds from which the oil is extracted (Aynechi, 1986). Its ripe seeds contain mixed fatty acids with a heigh content of mucilage while the leaves of this plant are consider rich in volatile oil (Overeem, 1999).

2. Materials and Methods

This study was performed in medicinal plants garden of Pharmacognosy and Medicinal Plants department of Pharmacy College of Baghdad University. The field experiment was carried out during the winter season of 2010-2011 , Balangu seeds were obtained from Herbal Medicine Center of Iraq and classified by National Herbarism of Agriculture Ministry , then

sown on November 10th in hilly. Field experiment was laid out in Randomized Complete Block Design (RCBD) with three replicates. The area of each plot (4m²) contained 4 rows. The distance between replicates was 2m. The soil is clay loam (63.2% clay, 37.7% silt, 1.1% sand) which contained 0.7 organic matters and had a pH of 7.5. The soil was tested by soil and water resources department of Agriculture College of Baghdad University. The experiment was contained 16 treatments prepared from four levels of N, P and K as soil application; (S0) without fertilizer; (S1) 300, 60 and 120; (S2) 150, 30 and 60 and (S3) 75, 15 and 30 kg. h^{-1} respectively along with four treatments of foliar application; (Fw) with water only as control; (F1) NPK (1000N + 500P + 1000K) and (F2) NPK (3000N + 1500P + 3000K) and (F3) NPK (5000N + 2500P + 5000) mg. L^{-1} .

Foiar application treatments were used with all soil application treatments. Soil fertilizers were added in three equal quantities at three times, the first application at cultivation; second application after 30 days, and third application after 65 day from cultivation. The nitrogen fertilizer was added as Urea (46% nitrogen), phosphorus fertilizer as super phosphate (21% P) while potassium fertilizer was added as potassium sulphate (45% K).

The plant samples were chosen from the middle rows of each plot randomly, the growth parameters as biometric indicator were included plant height, number of branches on plant and dry leaf weight (plate1) as shown in Figure 1.

Figure 1: Experimental cultivation of Belangu plant (*Lallemantia royleana*)

The volatile oil from leaves was extracted by steam distillation and the physical properties of volatile oil were determinate included specific gravity, density and refractive index by Guenther method (Guenther 1972).

All seed samples were powdered and placed in 72°C oven for 24h to be dried (Dini and Carapetiant, 2006) . The extraction with Ether method was used for measure of total oil content (Qutub, 1992). One gram of each samples were transferred in test tubes and 10 ml ether were added them, twice. Each time tubes were placed in 40°C oven for 12h and above solutions were transferred in balanced tubes. Tubes were placed in 40°C oven for 4h so that its other was evaporation. Weight of tubes before and after experience was used for oil content.

Fatty acids were determination by gas chromatography after the preparation of their methyl esters. This section of project was performed in siences and technolog ministry labrotary . Esterification was accomplished by addition of 3ml n-heptan in a test tube. The

tubes were vortexed for five min until the glycerol a supernatant. The amount of 0.2 ml from each sample was used for analysis.

The gas chromatography (Dany, Italy) model GC – 1000 equipped with a flame ionization detector and interface DS – 1000 integrator attached to a column for the separation of methyl esters was 30m long with 0/33 mm inner diameter. The column temperature was set from 100 to 220°C with an increment of 30°C/min for 3 min and following an 8 min stop at 180°C. it was again raised at rate of 10°C/min until the final temperature was reached. The injector and detector temperatures were set a 220°C.

All data recoded were subjected to analysis of variance and least significant difference (L.S.D) at 0.05 level implemented to compare the means of parameters studied (Steel and Torrie, 1960).

RESULTS

1- Biometric Parameters

The evaluation of the effect of soil and foliar N.P.K fertilization on plant height, number of branches per plant and leaf dry weight per plant as biometric parameters was occur in as shown in Table 1.

Table 1: Effect of Foliar and Soil N-P-K fertilization on plant height (cm)

F / S	Fw	F1 NPK	F2 NPK	F3 NPK	Average
S0	35	36	38	40	37.25
S1	44	53	60	65	55.5
S2	43	52	55	58	52.0
S3	43	45	53	54	48.75
Average	41.25	46.5	51.5	54.25	
L.S.d 0.05	S or F : (1.993), SxF: (3.987)				

The results were referred to significant effect by increasing the both soil and foliar fertilizers, at all treatments compared with control treatment, and the S1 (N300 , P60 , K120 kgha-¹) and S2 (N150 , P30 , K60 kgha-¹) treatments were gave significant increasing in plant height and gave the highest values were reached to 55.5 and 52.0 cm/plant with increasing percentage about 58.6% and 48.6% compared with control treatment (S0) without fertilizer which gave lowest value was reached to 35 cm/plant.

Also the plant height parameter was affected by increasing the foliar fertilization and the significant effect was obtained at all treatments compared the control treatment (spraying water). The highest values were obtained at F3NPK (N5000 + P2500 + K5000 megL^{-}) and F2NPK (N3000 + P1500 + K3000) treatments and treatments reached to 51.5 and 54.25

cm/plant respectively, the F3NPK and F2NPK were gave significant increasing reached to 26.1% and 19.8% compared with Fw treatment (spraying water) as control treatment.

The interaction treatments were gave significant effect with higher increasing in plant height by increasing soil and foliar NPK fertilization compared with S0 and Fw as control treatments. The highest value were obtained at S1F3NPK (N300 + P60 + K120 kgha¹ with N25 + P12.5 + K25 meg$L^{-1)}$ and reached to 65 cm/plant.

Table 2 show that soil and foliar NPK fertilization were gave significant effect on number of branches per plant, compared with control treatments. The S1 treatment was gave highest value and reached to 13.5 branch / plant with increasing percentage about 9.28% compared with S0 treatment (without fertilizer).

Table 2: Effect of Foliar and Soil N-P-K fertilization on Number of branches per plant

F / S	Fw	F1 NPK	F2 NPK	F3 NPK	Average
S0	5	7	8	8	7.00
S1	10	11	15	18	13.50
S2	9	11	14	15	12.25
S3	8	11	12	13	11.00
Average	8.00	10.00	12.25	13.50	
L.S.d 0.05	S or F : (1.391) , SxF : (2.782)				

The foliar fertilization by NPK fertilizers was gave significant effect on number of branches per plant and the F3NPK treatment was gave the highest value and reached to 13.50 branch / plant with significant increasing about 68% compared with Fw (foliar water) as control . In similar trend the significant effect was occurred in interaction treatments and the S1F3 NPK and S1F2 NPK treatments were gave the highest values were reached to 18 and 15 branch per plant for both treatments respectively.Leaf dry weight of the belangu plant was affected by soilar and foliar NPK fertilization and similar trend of other biometric was occurred as concluded in Table 3.

Table (3) Effect of Foliar and Soil N-P-K fertilization on leaf dry weight

F / S	Fw	F1 NPK	F2 NPK	F3 NPK	Average
S0	52	60	70	75	64.25
S1	90	130	158	165	135.75
S2	85	121	148	153	126.75
S3	80	95	138	143	114.00
Average	76.75	101.5	128.5	134.0	
L.S.d 0.05	S or F : (2.519) , SxF : (5.038)				

The highest value of leaf dry weight were obtained at S1 and S2 treatments as soil NPK fertilization and reached to 137.75 g / plant and 126.75 g / plant respectively .Also the F_3NPK as foliar fertilization were gave significant affect and obtained 134.0 and 128.5 g / plant respectively, with significant increasing about 74.6% and 67.4% compared with Fw treatment as control.

The S_1F_3NPK as interaction treatment was gave a highest value among all in interaction treatments and reached to 165 g / plant with significant increasing about 27% compared with control treatments.

2- Volatile oil study

Volatile oil percentage of the balangu leafs was significantly increased over that at control treatments and among soil NPK fertilizers treatment was gave highest value and reached to 2.16% while among the foliar NPK fertilizers treatments the F_3NPK was gave a highest value and reached to 2.14%. Among the interaction treatments the S_1F_3NPK treatment was gave a highest value and reached to 2.77% with significant increasing about 218% compared with SoFw as control treatment as shown in Table 4.

Table 4: Effect of Foliar and Soil N-P-K fertilization on Volatile oil percentage (%) of plant leaves

F / S	F w	F1 NPK	F2 NPK	F3 NPK	Average
S0	0.87	0.88	0.91	0.94	0.90
S1	1.21	1.93	2.75	2.77	2.16
S2	0.96	1.93	2.48	2.75	2.03
S3	0.95	1.30	1.98	2.11	1.58
Average	0.10	1.51	2.03	2.14	
L.S.d 0.05	S or F : (0.02430) , SxF : (0.04860)				

Density of volatile oil was increased with increasing soil and foliar NPK fertilization S_1 treatment as soil NPK fertilizer was gave highest value and reached to 0.924 meg / microliter while F_3NPK as foliar fertilizer was gave 0.918 meg / microliter and a highest value was obtained at S_1F_3NPK as interaction treatment and reached to 0.941 meg / microliter with significant increasing about 13.2% compared with SoFw as control treatments as shown in Table 5.

Table 5: Effect of Foliar and Soil N-P-K fertilization on Density (mg / microliter) of leaves volatile oil

F / S	Fw	F1 NPK	F2 NPK	F3 NPK	Average
S0	0.831	0.830	0.835	0.865	0.840
S1	o.896	0.920	0.938	0.941	0.924
S2	0.887	0.916	0.935	0.937	0.919
S3	0.885	0.898	0.927	0.930	0.677
Average	0.875	0.891	0.909	0.918	
L.S.d 0.05	S or F : (0.002684) , SxF : (0.005368)				

The similar trend of significant effect of both soil and foliar NPK treatment was obtained on specific gravity of volatile oil of plant leafs, S_1 and S_2 treatments were gave a heighest value among soil NPK fertilizer and reached to 0.974 and 0.972 respectively while the heighest values of foliar NPK fertilization were obtained at F_3NPK and F_2NPK treatments and reached to 0.974 and 0.972 also respectively as in Table 6.

Table 6: Effect of Foliar and Soil N-P-K fertilization on Specific gravity (mg / microliter) of leaves volatile oil

F / S	Fw	F1 NPK	F2 NPK	F3 NPK	Average
S0	0.962	0.963	0.965	0.966	0.964
S1	0.968	0.971	0.979	0.980	0.974
S2	0.967	0.970	0.974	0.976	0.972
S3	0.966	0.969	0.972	0.973	0.970
Average	0.966	0.968	0.972	0.974	
L.S.d 0.05	S or F : (0.1597) , SxF : (0.003193)				

The highest value of among interaction treatments was obtained at S_1 F_3NPK treatment and reached to (0.980 Meg / microliter) with significant increasing about 1.87% compared with SoFw as control treatments. The refractive index parameter of volatile oil was affected by increasing of soil NPK and F NPK fertilization and among soil NPK application the S_1 and S_2 treatment were gave a highest value and reached to 1.511° and 1.508° respectively while among foliar application a F_3NPK and F_2NPK were gave a highest value and reached to 1.509 and 1.510 respectively. The S_1F_3NPK was gave a highest value among interaction treatments and reached to 1.520 degree with significant increasing about 2.21%. Table 7 compared with SoFw as control treatment.

Table 7: Effect of Foliar and Soil N-P-K fertilization on Refractive index (degree) of leaves volatile oil

F / S	Fw	F1 NPK	F2 NPK	F3 NPK	Average
S0	1.487	1.489	1.489	1.490	1.488
S1	1.495	1.512	1.518	1.520	1.511
S2	1.493	1.510	1.515	1.516	1.508
S3	1.491	1.498	1.513	1.514	1.504
Average	1.491	1.502	1.509	1.510	
L.S.d 0.05	F or S : (0.002109) , FxS : (0.004217)				

3- Seed oil study

The results were refered to seed oil percentage was 34% and its contained five fatty acids include Linolenic acid (Lin) (56.37%) , Linolenic acid (L) (15.02%) stearic acid (S) (2.37%) Oleic acid (O) (14.83%) and palmitic acid (P) (7.91%) (fig1) & (fig2).

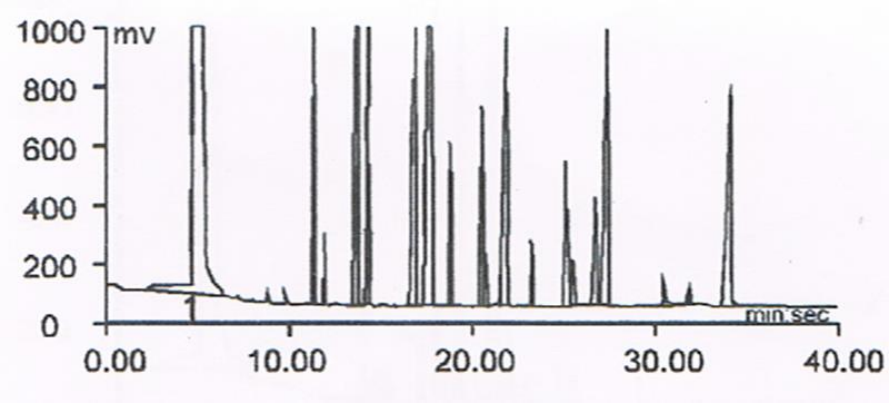

Figure 1: Chromatographically of standard

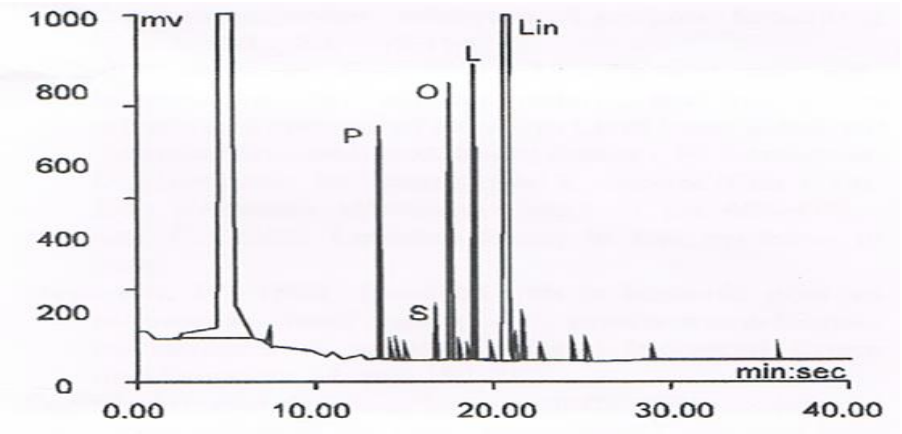

Figure 1: Chromatographically of Myandoab

Discussion:

The significant effect of both soil NPK and foliar NPK fertilization on all biometric parameters due to the balance of nutrient elements and gave more efficient for vegetative growth of plant with improving the root growth, this results were agreement with other study on chamomile plant (Ibrahim, 2007). Also the significant increasing of biometric parameters due to increasing activity of metabolism and phytosynthesis process (Blade and Rachid, 2001) .

The effect of NPK fertilizer by soil and foliar application may be belong to the nitrogen role in accumulation of clorophyle material which important in phytosynthesis process (Wander and Boumester, (2005) studied the influence of the phosphorus fertilizer on growth parameters and dry matter accumulation can be a scribed to its role in enhancing the nutrient up take by root system (Malka et al., 1998) . This study was agreement with same study on rosimery plant that gave highest leaf dry weight when treated with nitrogen fertilizer under semi- arid tropical condition (Romhold and Elfonly, 2000) . Generrally the results were indicated the nitrogen and phosphorus has appositive effect on the growth and physiology process of balangu plant .

From data of volatile oil at Table 4 were indicated the increasing of both soil and foliar NPK application leade to increasing the volatile oil quantity of leafes improving the physical properties , this efferet may be belonge to increasing the primary metabolism process such as photosynthesis that related with secondary metabolism products such as volatile oil (Handelmann and Dambroth, 1990) . Also improving the physical properties of volatile oil such as specific gravity , density and refractive index may be belonge to improvement the accumulation of oxygen compounds which solid compounds in belangu volatile oil (Azza et al., 2010) .

The increasing of percentage of volatile oil by increasing the soil and foliar NPK application due to the effect on the synthesis of tricycle – glycerol from glycerol - 3-phosphate which lead to mavelonic acid and to isoprene unit which is the building block terpenoid essential oil at the same time the aromatic ring which need the phosphorus fertilizer as building block of the two important moleculc phosphoral pyravic and erythrose-u-phosphate (Kandil, 2002) .

The data of seed oil were referes to different fatty acids with different percentage . The differences in fatty acids percentage of seed oil may belong to differences in environmental condition at locations study (Overeem, 1999) .

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